Environmental Assessment

for

Mosquito Management at
Benton Lake National Wildlife Refuge,
Great Falls, Montana

Lead Federal Agency: U.S. Department of the Interior
Fish and Wildlife Service

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Abstract:

This document describes the environmental effects of mosquito management by the Cascade County Weed and Mosquito Management District (CCMD) on Benton Lake National Wildlife Refuge in order to manage a documented threat to human health from West Nile virus. Mosquito management would involve treating up to 5,600 acres of wetland habitat with pesticides to kill mosquito larvae, and/or up to 6,800 acres of upland habitat with a broad-spectrum insecticide to kill adult mosquitoes. These pesticide treatments to reduce mosquito populations would occur only in response to a documented, mosquito-borne disease public health emergency involving refuge-based mosquitoes. The proposed (pesticide) treatment has been chosen as the preferred alternative. This EA also assesses the impacts of one other alternative on the local environment and public health.

Chapter 1
Purpose and Need for Action

1.1 PURPOSE AND NEED

This Environmental Assessment examines the environmental effects of emergency mosquito control at Benton Lake National Wildlife Refuge (NWR). The purpose of the proposed action is to manage a documented West Nile virus (WNV) health emergency. The Cascade County Weed and Mosquito Management District (CCMD) proposes to apply the mosquito larvicides *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Bs), or methoprene over a maximum of 5,600 acres of wetland habitat on the refuge, and/or the adulticides Anvil (sumithrin), Aqua-Reslin (permithrin) or Pyrenone (pyrethrin) over a maximum of 6,800 acres of upland habitat. The need for the proposed action is that the CCMD has documented that important vectors of WNV breed on the refuge and could contribute to an increasing risk for human infection. The proposed action would occur only when disease and mosquito population surveillance indicate an imminent threat to public health.

The CCMD is currently allowed to monitor mosquito populations on the Refuge, and this activity has been categorically excluded from National Environmental Policy Act analysis under Department of the Interior NEPA procedures for data collection and inventory (516 DM 2, Appendix 1.6; and 516 DM 6, Appendix 1.4 B[1]).

1.2 DECISION TO BE MADE

The decision to be made is whether to allow the CCMD to conduct mosquito control on Benton Lake NWR to protect public health in the event of a documented mosquito-borne disease health threat. This Environmental Assessment only examines the environmental effects of mosquito control measures conducted under situations of health emergencies, which by definition would imply that such measures would be infrequent and of short duration.

1.3 LEGAL MANDATES GOVERNING THE MANAGEMENT OF BENTON LAKE NWR

All National Wildlife Refuges are required to be managed for the purposes for which an individual refuge was established and to accomplish the mission of the National Wildlife Refuge System (NWRS). The mission of the NWRS as defined in the National Wildlife Refuge System Improvement Act of 1997 (NWRSIA) "is to administer a national network of lands and waters for the conservation, management, and where appropriate, restoration of fish, wildlife, and plant resources and their habitats within the United States for the benefit of present and future generations of Americans." The NWRSIA also directs that "the biological integrity, diversity, and environmental health of the System are maintained for the benefit of present and future generations of Americans."

Benton Lake National Wildlife Refuge was established by Executive Order in 1929 as a "Refuge and breeding ground for birds." The NWRSIA directs that before any use of a refuge can occur, that use must first be found to be compatible with the mission of the NWRS and the purposes for which the refuge was established. However, the Act does allow for exemptions to this compatibility standard when "necessary to protect the health and safety of the public or any fish or wildlife population".

1.4 ISSUES

The primary issues identified in this Environmental Assessment are: 1) the protection of human health from illness associated with West Nile virus; and 2) the potential for adverse environmental effects from intervention measures conducted to manage mosquito populations on the refuge.

1.4.1 West Nile Virus

West Nile virus (WNV) was introduced into North America in 1999 in the New York City area and has quickly spread across the continent. The virus is transmitted by mosquitoes, and is a disease that cycles primarily within bird populations. Humans and other mammals can become infected with WNV when bitten by an infectious mosquito. Although most human WNV infections are mild, approximately 1 in 150 people may develop meningitis or encephalitis, and of those that develop such serious illness, there is approximately 10-15% mortality. The elderly and persons with compromised immune systems are especially susceptible to developing serious illness from WNV. Horses are also susceptible to WNV infections and exhibit about a 30% mortality rate. An equine vaccine is currently available, but there is no vaccine currently available for humans.

The 2002 West Nile virus epidemic in the United States was the largest documented outbreak of mosquito-borne meningoencephalitis in the Western Hemisphere. Over 4100 cases of human West Nile meningoencephalitis and West Nile fever were documented, with nearly 300 deaths. Many thousands more were likely infected but suffered mild or no symptoms. In 2003, over 9800 clinical cases were identified in the United States, with 262 deaths. By 2004, the disease had spread from coast to coast in the U.S., with over 2500 documented human cases and 100 deaths.

Relatively few mosquito species are efficient at transmitting WNV. Several species in the genus *Culex* are the primary vectors of this disease, but species within other genera may become important vectors when virus activity becomes intense.

West Nile virus was first confirmed in *Culex* mosquitoes at Benton Lake National Wildlife Refuge in 2003. Human and equine cases, including fatalities were recorded within two miles of the Refuge boundary. Preparation of the Refuge Mosquito Control Strategy was completed in early 2004 in order to have a control protocol in place should West Nile once again be confirmed in Refuge mosquito populations.

West Nile virus is unique among other mosquito-borne viruses in the United States in that it causes substantial mortality in birds, particularly species in the family Corvidae (crows, jays, and magpies). Collection and testing of dead birds has become an important tool in detecting WNV activity. Virus activity is also monitored in adult mosquito populations by capturing mosquitoes in a trap, sorting them to species, and testing same-species pools for virus. Because WNV is primarily a disease of birds, sentinel chicken flocks are sometimes used to provide an initial indication of disease activity by regularly sampling the birds for WNV antibodies. WNV is also detected by testing symptomatic humans and horses.

A monitoring protocol has been established in conjunction with the CCMD. Sampling includes both adult and larval mosquitoes. The CCMD currently uses ABC adult mosquito traps to monitor WNV and mosquito populations on or near the refuge. A West Nile survey route has been established on the Refuge and is run twice weekly from June through October to recover and sample any freshly dead avian species to determine if West Nile virus was the cause of death. CCMD staff can sample on site and determine if West Nile virus is present. Threat levels and appropriate chemical treatment responses have been established based on the number of *Culex* mosquitoes that are captured since this species is the primary vector for West Nile virus.

1.4.2 Mosquito Management

Although there is currently no way to prevent all WNV infections, the Centers for Disease Control and Prevention (CDC) recommends controlling populations of mosquito vectors, particularly in the genus *Culex*, for minimizing human infections and managing an existing outbreak (CDC 2003). Mosquito management may involve breeding source reduction, the introduction or supplementation of predators of mosquito larvae, or the use of pesticides to kill larvae or adults.

Reducing the sources of mosquito breeding can involve both small- and large-scale measures. Many of the important vectors of WNV breed in artificial containers, so removing, draining, or treating such container habitats can reduce the risk of disease transmission. Larger scale source reduction involves the ability to manipulate water levels in managed wetlands to inhibit mosquito breeding.

Biological management of mosquito populations usually involves the introduction or supplementation of predatory fish into mosquito breeding habitats, with the goal that the fish will feed on larvae and provide a constant control measure. Both native and non-native species have been used for mosquito control.

Pesticides may be used to control either mosquito larvae or adults. Pesticides used to kill larval mosquitoes (larvicides) are applied directly to water. The two types of pesticides used most often to control larvae are *Bacillus* products and methoprene, an insect growth regulator. The two *Bacillus* products, *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus*, release toxins in the gut of mosquito larvae when ingested. Methoprene prevents the larvae from maturing to adults. Larvicides may be applied as liquids, granulations, or briquets, and can be applied manually or aerially.

Pesticides used to kill adult mosquitoes (adulticides) are broad-spectrum insecticides that are applied as a very fine (ultra-low volume–ULV) mist that kills adult mosquitoes as they fly. Adulticides may be applied by truck-mounted sprayers or aerially. There are two classes of adulticiding pesticides, organophosphates and pyrethrins. Organophosphates are cholinesterase inhibitors that affect the neuromuscular system. The two organophosphate adulticides most commonly used are malathion and naled. Pyrethrins may be either natural extracts of the chrysanthemum plant or synthetic versions thereof. The most commonly used pyrethrins are the synthetic pyrethroids permethrin, sumithrin, and resmethrin. All pyrethrins act on sodium channel functions in neural membranes. Synthetic pyrethroids are almost always paired with piperonyl butoxide (PBO), which acts as a synergist that blocks certain detoxifying enzymes in insects.

1.4.3 Other Measures to Reduce Disease Risk

Personal protection is an important element for reducing the human risk for mosquito-borne disease. Personal protection involves reducing exposure to mosquito bites by avoiding outdoor activities during evening hours when mosquitoes are most active, wearing protective clothing, and/or using insect repellent.

Chapter 2
Description of Alternatives

2.1 LIST OF ALTERNATIVES

This Environmental Assessment examines 2 alternatives: a pesticide alternative and a no-action (no pesticides) alternative. Both alternatives include monitoring of larval and adult mosquito populations.

2.2 THE NO-ACTION ALTERNATIVE

Under this alternative, no mosquito management would occur on the refuge. The Refuge would not allow the CCMD to apply any mosquito control pesticides to refuge lands. The CCMD would be allowed to monitor mosquito populations on the refuge, but no pesticide applications for mosquito control would take place within refuge boundaries.

Under this alternative, the Refuge would consider wetland management actions (such as draw-down) to decrease larval mosquito populations where *Culex* species exceed threshold levels, but would not permit larviciding or adulticiding to occur. During health emergencies, the refuge would consider closing all or part of the Refuge to visitors and restricting the outdoor activities of Refuge employees.

2.3 THE PROPOSED ACTION ALTERNATIVE

Under the proposed action, the refuge would allow the CCMD to apply mosquito control pesticides during periods of mosquito-borne disease health emergencies. Pesticides would be allowed only under the following conditions:

1) West Nile virus-positive *Culex* mosquitoes detected within five miles of Refuge, or Refuge located within published flight range(s) of virus-positive species <u>and</u> high levels (1-5 larvae per dip) of *Culex* larvae and adults ($\geq$ 90 female *C. tarsalis* or *C. pipiens* per 3 traps/night or $\geq$ 1,000 *Ochlerotatus spp.* or *Aedes spp*) are found in refuge marsh units.

Under these conditions, mosquito monitoring and dead bird surveillance would continue. The Refuge would consider wetland management actions (such as draw-down) to decrease mosquito populations where *Culex* species exceed threshold levels. Targeted larval and/or adult treatment would be applied (with Bti, *Bacillus sphaericus*, or methoprene, or a pyrethroid adulticide, respectively) based on the following evaluation supplied by surveillance data: 1) Does evidence indicate an increasing risk for human infection; 2)Are mosquito species found on the Refuge proven vectors of the disease; 3) Are the mosquito species found on the Refuge important bridge vectors; 4) Are the proven vector mosquito species found on the Refuge capable of flying far enough in large enough numbers to infect nearby residents; 5) Will treatment efforts lower the risk of disease to humans; and 6) Has a threshold level of vector species abundance been exceeded?

2) Virus-positive mammal-feeding species of mosquitoes found on Refuge marsh units or detected within five miles of Refuge or Refuge located within published flight range(s) of virus-positive species. Treatment thresholds for adult mosquitoes have been met or exceeded.

Under these conditions, mosquito monitoring and dead bird surveillance would continue. The Refuge would consider wetland management actions (such as draw-down) to decrease mosquito populations where *Culex* species exceed threshold levels, and continue larviciding if warranted through monitoring. Targeted adult treatment with a pyrethroid adulticide would be considered based on the answers to the following evaluation supplied by surveillance data: 1) Are mosquitoes found on the Refuge known vectors of the disease; 2) Are the mosquito species found on the Refuge important bridge vectors; 3) Are known vector mosquito species found on the Refuge capable of flying far enough in high enough numbers to infect nearby residents; 4) Will treatment efforts lower the risk of disease to humans; and 5) Has a threshold level of vector species abundance been exceeded?

3) Human or Equine cases of WNV have been detected within five miles of Refuge or Refuge located within published flight range(s) of virus-positive species, and virus-positive species found within the grassland units of the Refuge. Treatment thresholds for adult mosquitoes have been met or exceeded.

Under this scenario, mosquito monitoring and dead bird surveillance would continue. The Refuge would consider wetland management actions (such as draw-down) to decrease mosquito populations where *Culex* species exceed threshold levels, and continue larviciding if warranted through monitoring. Targeted adult treatment with a pyrethroid adulticide would be considered based on the answers to the following evaluation supplied by surveillance data: 1) Are mosquitoes found on the Refuge known vectors of the disease; 2) Are the mosquito species found on the Refuge important bridge vectors; 3) Are known vector mosquito species found on the Refuge capable of flying far enough in high enough numbers to infect nearby residents; 4) Will treatment efforts lower the risk of disease to humans; and 5) Has a threshold level of vector species abundance been exceeded?

Chapter 3
Affected Environment

Covering 12,383 acres, Benton Lake NWR is located on the western edge of the northern Great Plains, 50 miles east of the Rocky Mountains and 12 miles north of Great Falls, Montana. Benton Lake is actually a 5,000 acre shallow marsh that has been subdivided into 8 units by a system of dikes and water control structures. Natural runoff and precipitation is seldom adequate to maintain water levels in Refuge impoundments. Supplemental water is pumped to the Refuge from Muddy Creek, located fifteen miles to the west. Refuge impoundments are a closed system with no outlet and water must flow through the upper impoundments into the Interunit canal and finally into the lower units. The upper Units (1 and 2) are managed as semi-permanent marshes and hold water throughout the year. The lower units (3, 4a, 4b, 4c, 5 and 6) are managed as seasonal wetlands and are typically dry by mid-August. These units are reflooded between August and October to provide migratory bird habitat. Units 5 and 6 as well as part of Unit 4c are open for waterfowl hunting.

Native short grass prairie makes up most of the Refuge uplands with several hundred acres of seeded tame grasses and legumes.

During spring and fall migrations, up to 150,000 ducks, 2,500 Canada geese, 40,000 snow geese, 5,000 tundra swans and 50,000 shorebirds use the Refuge. Twenty Thousand ducks may be produced in a good year with more than 20,000 Franklin's gulls found on the Refuge. Avocets, black-necked stilts and white-faced ibis also nest on the Refuge. Of the approximately 240 species of birds recorded on the Refuge, nearly 90 are known to nest here.

Twenty-eight species of mammals, including white-tailed and mule deer, pronghorn, badgers, coyotes, cottontail and jackrabbits as well as skunks and raccoons can be found on the Refuge. No sizeable fish are found in Refuge impoundments, and a few reptile species, including garter snakes, bull snakes and prairie rattlers can be found.

Bald Eagles are sighted occasionally during spring and fall migration. No other listed or candidate species are found on the Refuge.

Recreation on the Refuge is geared toward wildlife-oriented activities such as wildlife observation, photography, and limited hunting for waterfowl and upland game birds. Students from all third grade classes in Great Falls visit the Refuge each spring to learn about birds and wetlands. An average of 10,000 visitors use the Refuge each year with the peak public use period occurring in late summer. Virtually all public use is confined to the Refuge Auto Tour and the hunting area during the fall.

Chapter 4
Environmental Consequences

The probable environmental consequences on selected resources for each alternative are presented in this chapter, providing the scientific and analytical basis for comparisons among the alternatives.

4.1 EFFECTS OF THE NO ACTION ALTERNATIVE

4.1.1 Effects on Habitat

Management of habitats within the refuge would continue as it has in the past. The No-Action Alternative would not result in any additional adverse effects on habitats within the Refuge.

4.1.2 Effects on Aquatic Resources

Management of aquatic resources within the refuge would continue as it has in the past. The No-Action Alternative would not result in any additional adverse effects on aquatic resources within the Refuge. Water level management could be used to control larval mosquitoes if it does not conflict with refuge purposes.

4.1.3 Effects on Wildlife

Because no mosquito control would occur, West Nile virus could potentially kill substantial numbers of birds.

4.1.4 Effects on Human Health

Because no mosquito control would occur on the refuge under any circumstances, this alternative could potentially threaten human health by exposing refuge employees, visitors, and neighbors to increased numbers of disease-carrying mosquitoes. Under these circumstances, personal protection measures such as protective clothing and insect repellent would be the primary defense against contracting a mosquito-borne disease.

4.2 EFFECT OF THE PROPOSED ACTION ALTERNATIVE

4.2.1 Effects on Habitats

Manually applied Bti and/or methoprene treatments would likely cause minor, short-term damage to vegetation from foot traffic immediately adjacent to wetland treatment areas. Vegetation damage would likely exacerbate damage caused during mosquito monitoring activities, but the damage would be limited to narrow corridors and vegetation would likely recover by the following season. Activities associated with applying Bti or methoprene aerially would not be expected to adversely affect habitats, as neither pesticide is known to be phytotoxic.

Applications of permethrin would be conducted with truck-mounted sprayers. Trucks would use existing roads and not adversely affect habitats. Permethrin is not known to be phytotoxic to vascular plants or algae.

4.2.2 Effects on Aquatic Resources

For the larvicides Bti, *Bacillus sphaericus*, and methoprene, there may be potential adverse effects to aquatic invertebrates. All adulticides may adversely affect aquatic invertebrates and fish. There is less known about potential impacts to amphibians and reptiles from mosquito control pesticides.

4.2.2.1 Effects of Bti. Like other varieties of the natural soil bacterium, *Bacillus thuringiensis* (Bt), Bti is a stomach poison that must be ingested by the larval form of the insect in order to be effective. Bt contains crystalline structures containing protein endotoxins that are activated in the alkaline conditions of an insect's gut. These toxins attach to specific receptor sites on the gut wall and, when activated, destroy the lining of the gut and eventually kill the insect. The toxicity of Bt to an insect is directly related to the specificity of the toxin and the receptor sites. Without the proper receptor sites, the Bt will simply pass harmlessly through the insect's gut. Several varieties of Bt have been discovered and identified by the specificity of the endotoxins to certain insect orders. *Bacillus thuringiensis* var. *kurstaki*, for example, contains toxins that are specific to lepidopterans (butterflies and moths), while Bti is specific only to certain primitive dipterans (flies), particularly mosquitoes, black flies, and some chironomid midges. Bti is not known to be directly toxic to nondipteran insects.

Because Bti must be ingested to kill mosquitoes, it is much more effective on first-, second-, and early third-instar larvae than on late third and fourth instars since the earlier instars feed at a faster rate (fourth instar larvae feed very little). The pesticide is completely ineffective on pupae because they do not feed at all. Formulated products may be granular or liquid, and potency is expressed in International Toxicity Units (ITU), usually ranging from 200-1200 ITU. The concentrations of Bti in water necessary to kill mosquito larvae vary with environmental conditions, but are generally 0.05-0.10 ppm. Higher concentrations (0.1->0.5 ppm) of Bti are necessary when there is a high amount of organic material in the water, late-third and early fourth instar larvae predominate, larval mosquito density is high, or water temperature is low (Nayar et al. 1999). Operationally, Bti is applied within a range of volume or weight of formulated product per acre as recommended on the pesticide label, with the goal to achieve an effective concentration. The label recommended range of application rates under most conditions varies by a factor of 4 for most formulations (e.g., for granular formulations, 2.72-11.12 kg/ha [2.5-10 lb/acre]). For later instar larvae and water with a high organic content, higher application rates are recommended that may reach 8 times the lowest rate (e.g., for granular formulations, the higher rate is 11.1-22.5 kg/ha [10-20 lb/acre]). Mosquito control agencies use the recommended label rates, along with previous experience, to administer an effective dose. Typical application rates are often in the mid- to upper values of the normal ranges recommended on the labels (Abbott Laboratories 1999). Because water depths even within a single wetland can vary greatly, field concentrations of Bti can vary widely, especially when the pesticide is applied aerially. Efficacy is monitored by post-application reductions in mosquito larval density, but the actual concentration of Bti following an application is not measured. Thus, an insufficient concentration of Bti can be detected by low mortality of mosquito larvae, but an overdose (i.e., a concentration greater than necessary to kill mosquito larvae) of the pesticide is rarely monitored.

The issue of Bti concentration is important with regard to impacts on nontarget organisms. Of particular concern is the potential for Bti to kill midge larvae (family Chironomidae). Chironomid (non-biting midge) larvae are often the most abundant aquatic insect in wetland environments and form a significant portion of the food base for other wildlife (Batzer et al. 1993; Cooper and Anderson 1996; Cox et al. 1998). Negative impacts on chironomid density/biomass could have deleterious effects on wetland/wildlife food webs and could also lower biodiversity.

The potential for Bti to impact chironomid populations depends on the fate and availability of the pesticide, the ingestion of the pesticide, and the presence and number of specific receptor sites in the insect gut for the toxins (as discussed above). Fate and availability encompass both the initial dose/concentration and the fate of the pesticide in the aquatic environment. Chironomid larvae live primarily in the benthos of wetlands. Mosquito larvae ingest Bti primarily within the water column, but Bti readily adheres to suspended particulate matter and settles to the benthos (Yousten et al. 1992).

Ingestion of Bti by chironomid larvae depends primarily on the feeding mechanism. The family Chironomidae is a relatively large group, with nearly 1,000 species identified for North America (Merritt and Cummins 1996). This family encompasses a variety of feeding strategies: filter-feeders, collector-gatherers, scrapers, shredders, and even predators. Filter-feeding larvae are more likely to ingest Bti than larvae with other feeding strategies (Pont et al. 1999).

Chironomid larvae appear to possess mid-gut receptor sites for Bti endotoxins similar to those in mosquito larvae, and exhibit similar histopathological changes in the gut lining that lead to death of the insects when exposed to lethal concentrations of the pesticide (Yiallouros et al. 1999). There are, however, differences in the susceptibility of midge larvae to Bti at the subfamily level and among larval instars. In general, larvae in the subfamily Chironominae (Tribes Chironomini and Tanytarsini) are more susceptible to Bti than larvae of other subfamilies (Ali 1981; Pont et al. 1999; Yiallouros et al. 1999). Also, early-instar larvae are much more susceptible to Bti than later instars (Ali et al. 1981; Charbonneau et al. 1994).

There have been a number of laboratory and field studies examining the toxicity of Bti to chironomid larvae (Boisvert and Boisvert 2000). There have been many different formulations and potencies of Bti products used in these studies, and in many cases actual concentrations of Bti within the water were not measured. Also, differences in the species and instar of the chironomid larvae used (sometimes not specified), and in the environmental conditions of the field experiments make direct comparisons among the studies difficult. Most field studies examined the nontarget effects from a single application of Bti and did not address the potential long-term impacts from repeated applications over a season or over several seasons.

It is clear that in laboratory studies Bti is lethally toxic to some species of chironomid larvae at concentrations expected for mosquito control. Charbonneau et al. (1994) determined an EC_{50} (the concentration required to cause an effect in 50 percent of the test population) of 0.20 ppm for *Chironomus riparius* (fourth instar), and the toxicity of Bti to earlier instars was over two orders of magnitude greater. Similarly, Ali et al. (1981) found the LC_{50} (the concentration required to kill 50 percent of the test population) for first-instar *Glyptotendipes paripes* (0.034 ppm) to be over two orders of magnitude lower than the LC_{50} for third instar larvae (8.31 ppm).

Charbonneau et al. (1994) studied the effects of Bti on chironomid larvae in the laboratory and the field. Laboratory toxicity tests on Chironominae larvae (the most susceptible subfamily) demonstrated up to 100 percent mortality at label-recommended rates, but the toxicity of Bti to chironomids was influenced by several environmental factors. Factors that lowered toxicity to chironomids included higher water temperature, greater water depth, organic matter, and coverage by macrophytes. Field enclosure tests with Bti applied at 5.6 kg/ha (5 lb/acre) failed to demonstrate any pesticide effects on midge larvae within the enclosures, leading the authors to conclude that environmental factors reduce the toxicity of Bti to chironomids in the field. However, mortality of nontarget organisms within the enclosures was measured after 48 hours. Apparent effects of Bti on chironomids may not be detectable for 5-7 days post application (Ali 1981; Lacey and Mulla 1990; Pont et al. 1999). Also, because early instar larvae are much more susceptible to Bti, first and second instars would likely exhibit the greatest mortality. The 575 μm mesh used to sample benthic invertebrates in the field tests of the Charbonneau et al. (1994) study, however, was too large to effectively sample first- and some second-instars. Thus, the conclusions regarding the field component of this study must be viewed with caution.

There is some evidence from field studies in which negative impacts to chironomid larvae were observed that such impacts are relatively short-lived (e.g., Miura et al. 1980). In most of these studies, however, it is not clear if the rebounding densities of midge larvae represent the same species or even the same subfamily that was initially reduced by the pesticide. Furthermore, population-level impacts to species from repeated applications over a season were usually not addressed. Although many species of chironomids are capable of producing several generations per year and could re-colonize a treated wetland relatively quickly, other species have only one generation per year and therefore would not be able to re-colonize until the following year. The ability of Bti-susceptible species to re-colonize a wetland following pesticide treatment would also depend on 1) the frequency of Bti applications, 2) the extent of Bti treatments within the wetland, and 3) the extent of Bti applications in the surrounding landscape. Widespread larviciding with Bti would provide few refugia for re-colonizing source populations of susceptible species.

In a study that examined population-level impacts to chironomids from a single application of Bti at a mosquito control rate, investigators showed that, while there was no statistical difference in the number of emerging adult chironomids between control and treatment enclosures, the species composition was different (Pont et al. 1999). Species sensitive to Bti (*Tanytarsus horni, T. fimbriatus, and Microchironomus deribae*) were 24-54 percent less abundant in enclosures treated at mosquito control rates than in control enclosures, while a less sensitive species (*Polypedilium nubifer*) was over 200 percent more abundant in the treated enclosure versus control. Higher application rates resulted in greater reductions of the Bti-sensitive species. This suggests that as Bti-sensitive chironomid larvae are killed by the pesticide, less sensitive species may thrive as they are released from competition (Pont et al. 1999). Thus, although chironomid larval numbers often appear to rebound after a treatment with Bti, this may be indicative of a shift in the species composition of the community, with species less sensitive to Bti replacing the sensitive species. It is unknown how or if such a shift would affect food web dynamics, but biodiversity would be lowered.

There is only one published study that examined the long-term, nontarget effects of Bti (Hershey et al. 1998; Niemi et al. 1999). In this study conducted in Minnesota, 27 wetlands were sampled for macroinvertebrates over a 6-year period. All wetlands were sampled for 3 pre-treatment years and randomly assigned to 3 treatment groups: Bti, methoprene (see discussion below), and an untreated control group. The wetlands were sampled for 3 treatment years. Bti was applied to wetlands in a granular formulation at the rate of approximately 11.1 kg/ha (10 lb/ac), which represents the high end of the normal label-recommended application range. Bti was applied to each treatment wetland 6 times per year at intervals of 3 weeks or after rainfall of >1.25 cm, whichever came first (Niemi et al. 1999). Although this frequency of application is high, it is within the range that could occur from operational mosquito control.

After the first year of treatment, no differences in macroinvertebrate density, biomass, or community composition (richness of genera) among the treatments were observed (Hershey et al. 1998). However, in the second and third years of treatment, highly significant differences were observed in the two treatment groups compared to control. Chironomid larvae were significantly impacted by Bti treatments, with reductions in density of 66 percent and 84 percent for the second and third years of the study, respectively, compared to densities in control wetlands (Hershey et al. 1998). Significant declines in other nematoceran (primitive) dipteran larvae were also observed during the last two years of the study. There were also declines in macroinvertebrate predator densities in the Bti treated wetlands that the authors interpret as indirect effects from the reduction in a prey base dominated by chironomid larvae (Hershey et al. 1998; Niemi et al. 1999).

There is clear evidence from both laboratory and field studies that Bti can kill some chironomid larvae. Species in the subfamily Chironominae are apparently the most susceptible to direct toxicity; other subfamilies exhibit little mortality at mosquito control rates. Even within the subfamily Chironominae there are apparent differences among in susceptibility to Bti, relating perhaps to feeding mode (Pont et al. 1999). Within susceptible species, toxicity is greatest to early instars. Lethal concentrations of Bti are orders of magnitude lower for early versus late instars, and well within the concentrations expected from operational mosquito control. There is evidence that environmental conditions such as temperature, organic content of the water, vegetation, and density of larvae can ameliorate some of the potential negative impacts to chironomid larvae (Charbonneau et al. 1994), although field experiments designed to test this may be suspect.

The only long-term study on the nontarget effects of Bti for mosquito control demonstrated significant adverse effects on the chironomid community of treated wetlands, and this translated into numerous significant negative effects within the food web (Hershey et al. 1998; Niemi et al. 1999). The intensity of Bti applications used in this study, both the application rate and the frequency of applications, would represent the high end of those that would normally occur for operational mosquito control. In addition, entire wetlands were treated, which may or may not occur with aerial applications of Bti. Thus, the Minnesota study may represent a "worst-case scenario" of potential mosquito control operations, but it has generated the only data available on the long-term nontarget effects from Bti. Studies that examine nontarget effects of Bti from a single application or even within a single season may not be adequate to detect potential long-term impacts from pesticide use (Hershey et al. 1995).

There is also evidence that application rate can have a profound effect on impacts to chironomids from Bti (Rodcharoen et al. 1991). Because application rates of Bti for mosquito control can vary by a factor of 8, field concentrations of the pesticide can reach levels that are toxic to chironomid larvae, yet are still within the pesticide label directions. In addition, there are no label restrictions on the number of applications of Bti to any one area. Economic considerations may preclude regular applications at the highest label rate, yet even at lower rates, adverse impacts to chironomid midge larvae have been demonstrated (Miura et al. 1980; Ali 1981; Ali et al. 1981).

In addition to the potential adverse impacts to aquatic invertebrate communities, one study has demonstrated that Bti may be toxic to some species of algae. Su and Mulla (1999) showed significant declines in densities, compared to controls, of the algal species *Closterium* and *Chlorella* following treatments with two formulations of Bti in experimental microcosms.

4.2.2.2 Effects of *Bacillus sphaericus*. *Bacillus sphaericus* (Bsph) is a naturally-occurring soil bacterium similar to Bti, and has been developed as a commercially-available mosquito larvicide since the early 1990s. Like Bti, it releases a protein endotoxin in the alkaline gut of larval mosquitoes that attaches to specific receptor sites of susceptible species. This endotoxin dissolves the lining of the gut wall and eventually kills the larva. Unlike Bti, Bsph has only one endotoxin (Bti has two or more). Also, unlike Bti, Bsph is very effective in water with a high organic content, and is therefore often used in such habitats for control of certain *Culex* mosquitoes. Bsph is also capable of "cycling" in the aquatic environment, meaning it can retain its larvicidal properties after passing through the gut of a mosquito and—unlike Bti—provide effective mosquito control for weeks after a single application. Bsph, however, is not effective on all species of mosquitoes.

Because Bsph is a more recently developed larvicide than Bti, there are fewer studies that have examined the nontarget effects of this pesticide. The data available, however, indicate a high degree of specificity of Bshp for mosquitoes, with no demonstrated toxicity to chironomid larvae at any mosquito control application rate (Mulla et al. 1984; Ali and Nayar 1986; Lacey and Mulla 1990; Rodcharoen et al. 1991). This high specificity to some mosquito species and low toxicity to chironomid larvae is probably the consequence of the one endotoxin contained with the Bsph spore. Unfortunately, this also makes the development of resistance to this pesticide more likely if this larvicide becomes widely and frequently used.

A water dispersible granular formulation of *Bacillus sphaericus* has been found to reduce densities of the algal species *Closterium* and *Chlorella* (Su and Mulla 1999) in experimental microcosms.

4.2.2.3 Effects of Methoprene. Methoprene is a synthetic mimic of a naturally produced insect hormone, juvenile hormone (JH). All insects produce JH in the larval stages, with the highest levels occurring in the insect's early developmental stages. As an insect reaches its final stage of larval development, the level of JH is very low. This low level of JH triggers the development of adult characteristics. When an insect is exposed to methoprene, a hormonal imbalance in the development of the insect results, and it fails to properly mature into an adult. The insect eventually dies in the pupal stage. The most susceptible stages of development to methoprene are the later instars (for mosquitoes, third and fourth instars). In mosquito control applications, methoprene is applied directly to the larval breeding habitat. Larvae will continue to feed and may reach the pupal stage, but they will not emerge as adults. Methoprene is completely ineffective on mosquito pupae and adults. It is available in several formulations: liquid, granular, pellet, and briquet. There are several micro-encapsulated and extended-release formulations that remain effective for up to 150 days.

The amount of methoprene necessary for mosquito control is < 1.0 part per billion (ppb). The initial concentrations of methoprene when applied to aquatic habitats may reach 4-10 ppb, but residual concentrations are approximately 0.2 ppb (Ross et al. 1994). Once released into the aquatic environment, it is non-persistent, with a half-life of about 30-40 hours. Micro-encapsulated and extended-release formulations will, of course, be present in the water longer as the pesticide is slowly released over time, 7-150 days, depending on the formulation. In field applications, efficacy is determined only by an observed inhibition of emergence of adults, since larvae are not directly killed by the pesticide.

Because methoprene is a JH mimic and all insects produce JH, there is concern about potential adverse impacts to nontarget aquatic insects when this pesticide is used for mosquito control. As with Bti, there is particular concern regarding potential negative impacts to chironomid larvae due to their importance in food webs. As with any pesticide, toxicity is a factor of dose plus exposure.

At mosquito control application rates, methoprene is present in the water at very small concentrations (4-10 ppb, initially). With regard to exposure, chironomid larvae occur primarily in the benthos, either within the sediments and/or within cases constructed of silk and detritus. Thus, there may be differences with regard to exposure to methoprene between chironomid and mosquito larvae, the latter occurring primarily in the water column.

The published literature on the impacts of methoprene to chironomids is not as extensive as that for Bti. However, there is evidence for potential toxicity to chironomid and other aquatic invertebrates from methoprene treatments. Some early experiments indicated approximately 50 percent mortality of *Chironomus stigmaterus* (Chironomidae) and 70 percent of *Brachydeutera argentata* (Diptera: Ephidridae) larvae when exposed to 0.01 ppm of technical grade methoprene (Miura and Takahashi 1973). Mulla et al. (1974) noted up to 100 percent inhibition of emergence for some midge species, although the lowest concentration tested was 0.1 ppm. Breaud et al. (1977) observed reductions in several aquatic invertebrate taxa, including chironomids, after six applications of methoprene over an 18-month period in a Louisiana marsh. The application rate in this latter study was 0.028 kg/ha of active ingredient, although the formulation was not specified (Breaud et al. 1977).

In testing different formulations of methoprene against chironomids in experimental ponds, Ali (1991a) found that sustained-released formulations inhibited emergence of midges by 38-98 percent, in some cases for up to 7 weeks. A liquid, microencapsulated formulation applied at mosquito control rates resulted in a 60 percent inhibition of emergence in the tribe Chironomini for 14 days post-treatment. A pelletized, sustained-release (30 days) formulation applied at mosquito control rates inhibited all chironomid emergence by 64-98 percent for 7 weeks. A briquet formulation (30 days sustained-release) produced 38-98 percent inhibition of all chironomids for 7 weeks. The granular formulation applied at the high end of mosquito control rates reduced chironomid emergence by 61-87 percent (Ali 1991a).

In the multi-year Minnesota study cited above in the section on Bti, a 3-week sustained-release, granular formulation of methoprene was applied to treatment wetlands at a label-recommended rate of 5-10 kg/ha (Hershey et al. 1998; Niemi et al. 1999). The pesticide was applied six times per season at 3-week intervals. The impacts from methoprene in this study were very similar to those observed for Bti. Negative impacts were not observed until the second and third years of treatment. In those years, significant declines in aquatic insect density and biomass were detected in methoprene-treated wetlands compared to controls. Total insect biomass was 70 percent and 81 percent lower in the second and third years of treatment, respectively, than in control wetlands (Hershey et al. 1998). Reductions were observed across many insect taxa, including predators and non-predators, suggesting direct (pesticide) and indirect (food web) effects from methoprene treatments (Hershey et al. 1998).

Although the application rate of methoprene used in the Minnesota study was well within operational rates used in mosquito control, the frequency of application exceeded what would probably occur under most field situations. Using a 3-week sustained release formulation and applying that every 3 weeks ensured a nearly constant exposure of methoprene to aquatic invertebrates in the treated wetlands throughout the season. Under such a scenario, it is unlikely that most impacted invertebrate populations would be able to re-colonize the wetlands during the treatments. However, this does not discount the conclusion that nontarget aquatic invertebrates were indeed impacted by methoprene at rates and concentrations used for mosquito control. Whether or not the observed food web effects would have been lessened under a more realistic pesticide application regime is debatable.

Studies of adverse impacts from methoprene on insect taxa other than chironomids are less conclusive. Because methoprene affects insect development and does not directly kill larvae, traditional toxicity testing over a few days is often inadequate when looking for potential impacts. Methoprene toxicity can only be observed at the point in which the immature insects reach (or fail to reach) adulthood. Thus, many published laboratory and field studies looking at nontarget impacts from methoprene were of insufficient duration to detect actual negative impacts (e.g., Miura and Takahashi 1973).

Breaud et al. (1977) observed adverse effects from methoprene on 14 aquatic invertebrate taxa, including *Callibaetis* sp. mayflies, odonates (dragonflies and damselflies), predaceous diving beetles, and chironomids. Negative impacts to *Callibaetis* mayflies from methoprene treatments have been observed by others (Steelman et al. 1975; Norland and Mulla 1975). Miura and Takahashi (1973) did not observe any mortality on *Callibaetis* from methoprene in laboratory or field studies, but neither was of sufficient duration (48 hours and 1 week, respectively) to adequately detect developmental effects (Miura and Takahashi 1973). Pinkney et al. (2000) observed consistently lower numbers of mayflies emerging from methoprene-treated wetlands compared to controls, but these differences were not statistically significant (Pinkney et al. 2000).

There is evidence of methoprene impacts to non-insects as well. McKenney and Celestial (1996) noted significant reductions in number of young produced in mysid shrimp at 2 ppb (McKenney and Celestial 1996). Sub-lethal effects on the cladoceran, *Daphnia magna*, in the form of reduced fecundity, increased time to first brood, and reduced molt frequency have also been observed at concentrations < 0.1 ppb (Olmstead and LeBlanc 2001).

There has been speculation and some preliminary data to suggest that methoprene causes limb malformations in amphibians (La Clair et al. 1998). However, experiments with methoprene and its degradation products have failed to demonstrate developmental toxicity even at concentrations exceeding 100 times that expected for mosquito control (Ankley et al. 1998; Degitz et al. 2003). Therefore, current data do not support a role of methoprene in amphibian malformations.

In summary, there is evidence for significant adverse nontarget effects from methoprene even when applied at mosquito control rates. With regard to negative impacts to chironomid midges, there may be differences in susceptibility among species and differences depending on the formulation used. One study in particular suggested that methoprene formulations with short-term residual activity may have smaller impacts to chironomids (Ali 1991a). However, even the "ineffective" liquid formulation used in this study reduced emergence of Chironomini midges by 60 percent for two weeks. Certainly, not all midges will be affected by a single application of methoprene for mosquito control. However, the apparent differences in pesticide formulations, the varied susceptibility of species, and perhaps even the influence of some as-yet-undetermined environmental factors, make predicting the degree of any impacts nearly impossible.

Because methoprene does not immediately kill susceptible chironomid larvae, they are still available for predators. However, repeated applications of methoprene over a mosquito breeding season would eventually hinder recruitment as adults repeatedly fail to emerge (Hershey et al. 1998). Longer-term studies conducted over the course of a season or over multiple seasons are especially necessary for examining nontarget impacts from methoprene in order to detect potential impacts on longer-lived larvae (e.g., odonates, mayflies, and aquatic beetles) and to detect potential impacts to long-term recruitment. As was the case with Bti, the ability for a population to re-colonize a wetland following a methoprene treatment would depend on the intensity and frequency of applications at different spatial scales.

4.2.2.4 Effects of Pyrethroid Adulticides. All pesticides used to kill adult mosquitoes are broad-spectrum insecticides. The only selective aspect of these pesticides is in the manner in which they are applied. Most adulticides used currently are applied as ultra-low volume (ULV) sprays, meaning relatively small amounts are used (compared to some agricultural pesticides) and they are sprayed as very fine droplets (10-30 μm in diameter). This small droplet size allows the spray to drift for a relatively longer period of time compared to larger droplets, and the small size delivers an appropriate dose of the pesticide to kill an adult mosquito. Drift is a necessary component of adulticiding because these sprays are most effective on flying insects. For this reason, adulticide applications generally occur in the evening or early morning hours when the majority of mosquito species are most active.

Pyrethroid insecticides are usually combined with the synergist piperonyl butoxide (PBO), which interferes with an insect's detoxifying mechanisms and increases the efficacy of these pesticides. Pyrethroids have low to moderate acute mammalian toxicity, practically no acute avian toxicity, extreme fish toxicity, and super aquatic invertebrate toxicity (Siegfried 1993). For permethrin, the 96-hour LC_{50} (concentration that would kill 50% of the population) for the cladoceran, *Daphnia magna* is 0.039 ppb (parts per billion). The 48-hour LC_{50} for the midge larva, *Chironomus plumosus* is 0.56 ppb. For fish, the 48-hour LC_{50} for bluegill sunfish is 1.8 ppb. For sumithrin, the LC_{50} for *Daphnia magna* is >300 ppm (parts per million) and 15.8 ppb for bluegill sunfish. The actual toxicity of pyrethroids such as permethrin in aquatic habitats, however, is less than may be anticipated because of the propensity of these pesticides to adsorb to organic particles in the water (Hill et al. 1994). There are also data that indicate synthetic pyrethroid degradates have endocrine disrupting properties (Tyler et al. 2000).

All adulticides are very highly toxic to aquatic invertebrates in concentrations > 1 ppb (Anderson 1989, Milam et al. 2000). Because most adulticides can be applied over or near water when used for mosquito control, there are risks to aquatic invertebrates from direct deposition and runoff of the pesticides. However, very few field studies have been conducted that have examined the impacts to aquatic organisms from mosquito control adulticides. The limited number of studies on adulticide impacts all involve examining short-term effects, usually from a single application of a pesticide. Therefore, it is difficult to extrapolate the results of short-term experiments into predictions of long-term impacts, whether the short-term studies detected impacts or not. In addition, mosquito control is most often conducted at a landscape level. Studies of impacts at such larger temporal and spatial scales are non-existent, and would be a challenge both scientifically and economically.

If permethrin drift enters Refuge waters, there is the potential to adversely affect aquatic invertebrate and fish. Fish could be affected directly by the toxicity of the pesticide, or indirectly if the invertebrate food base is diminished by direct toxicity. Adulticides will not be applied within 100 feet of Refuge wetlands to reduce the amount of pesticide entering Refuge waters.

4.2.3 Effects on Wildlife

The larvicides Bti, *Bacillus sphaericus*, and methoprene are not anticipated to have direct adverse effects on terrestrial wildlife, due to their low toxicity to mammals and birds. Applications of these pesticides manually or aerially could temporarily disturb wildlife, including nesting birds, but are not anticipated to have any long-term effects.

While applications of pyrethroid adulticides are not expected to result in any acute or chronic toxicity to mammals or birds, there is a high probability of mortality to terrestrial insects because this is a broad-spectrum insecticide (Jensen et al. 1999). There are data indicating the high toxicity of adulticides to honey bees (Taylor et al. 1987; Hagler et al. 1989; Pankiw and Jay 1992a; Pankiw and Jay 1992b), although the timing of adulticide applications in the evening can be expected to minimize these impacts. The timing of applications, however, will not minimize the adverse effects of this pesticide on insects that are active during the applications. Salvato (2001) examined the toxicity of naled, malathion, and non-synergized permethrin to 5 species of butterflies, including larval and adult stages. Naled and permethrin were found to be the most toxic to all life stages. The LD_{50} data presented for some larvae and adults coincide with that delivered by a single ULV droplet of 5-23 μm, within the desired range for mosquito control (Salvato 2001). Mosquito control adulticiding has been identified as a likely contributing factor in the decline of several rare lepidopteran species in the Florida Keys (Calhoun et al. 2000; Salvato 2001).

A reduction in terrestrial insect biomass could result in secondary adverse impacts to wildlife, such as insectivorous birds, that depend on insects for food. Because such pyrethroid applications are expected to be infrequent and of short duration, long-term adverse effects on non-target insects or indirect effects on other wildlife are not anticipated.

4.2.4 Effects on Human Health

Applications of Bti, *Bacillus sphaericus*, or methoprene are not expected to adversely affect human health. All of these larvicides are relatively non-toxic to humans, but sensitive individuals could experience an allergic reaction. Application of larvicides will be undertaken by trained personnel of the CCMD in wetland habitats away from any residences. Pyrethroids have low mammalian toxicity, and the rates used for mosquito control (.0012 lbs. AI/acre to .007 lbs. AI/Acre) should not result in acute toxicity to humans. However, sensitive individuals could experience a reaction to the pesticide if exposed. Benton Lake NWR will be closed to all public access if it becomes necessary to apply adulticides on the Refuge. The Refuge will remain closed for 24 hours to ensure that no one, including staff, is exposed to potentially harmful chemicals.

4.3 COMPARISON OF ENVIRONMENTAL CONSEQUENCES

4.3.1 Environmental Effects of the No Action Alternative

Under this alternative, no mosquito control would occur on the refuge. No direct effects would occur on refuge resources from pesticides or water management activities. Disease-carrying mosquitoes could potentially threaten the health and safety of refuge employees, visitors, and wildlife, and could move from the refuge to threaten the health of off-refuge neighbors and domestic animals.

4.3.2 Environmental Effects of the Proposed Action Alternative

Under this alternative, the CCMD would apply the larvicides, Bti, *Bacillus sphaericus* or methoprene to wetlands within the Refuge identified as *Culex* breeding areas through surveillance, or a pyrethrin adulticide, to upland areas of the Refuge when there is a documented mosquito-borne disease health threat. Equipment used to apply these pesticides would use existing roads and would not damage wildlife habitats. Bti, *Bacillus sphaericus*, and methoprene may temporarily reduce the number of invertebrates within treated wetlands. Given that these treatments will only occur in the case of a documented mosquito-borne disease health threat, they are expected to be infrequent, and thus not result in any long-term reductions in the invertebrate food base. Spraying with pyrethroid adulticides would occur a minimum of 100 feet from Refuge wetlands, which will reduce the amount of pesticide that could adversely affect aquatic organisms. Pyrethroid spraying may temporarily reduce the number of terrestrial flying insects, but since such pesticide treatments will occur infrequently and only in small areas of the refuge, no long-term or wide-spread reductions are expected. Bti, *Bacillus sphaericus*, methoprene, and pyethroids are not persistent in the environment.

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